AMENDMENTS TO THE SPECIFICATION

IN THE ABSTRACT OF THE DISCLOSURE:

Replace the Abstract of the Disclosure currently of record with the attached new Abstract of the Disclosure.

IN THE SPECIFICATION:

Please amend paragraph [0008] as follows:

[0008] The present invention relates to a process for producing a peptide or a peptide derivative by using a reaction system of transcribing a DNA into an RNA and then translating the RNA produced or a reaction system of translating an RNA *in vitro* wherein ~~characterized in that~~ a part or all of protein components constituting the transcription/translation reaction system are labeled with one of a pair of substances adhering to each other and the other substance is used as an adsorbent for capturing said labeled protein components after translating. In this process, a plural number of combinations of said first and second substances ~~the substance~~ used for labeling a part or all of the protein components constituting the reaction system with the substance used as an adsorbent for capturing the labeled protein components may be used in the transcription/translation reaction system.

Please amend paragraph [0009] as follows:

[0009] The protein components labeled with a first substance or a second substance ~~one of a pair of the substances~~ adhering to each other are a part or all of factors and enzymes for the transcription or translation reaction. Particular examples of these factors and enzymes include initiation factors, elongation factors, termination factors, aminoacyl-tRNA synthetase, methionyl-tRNA transformylase and RNA polymerase.

Please amend paragraph [0010] as follows:

[0010] The protein components labeled with a first substance or a second substance ~~one of a pair of the substances~~ adhering to each other are the factors and enzymes for the transcription or translation reaction and other enzymes required in the constitution of the reaction system. Examples of the enzymes required in the constitution of the reaction system other than the factors and enzymes for the transcription or translation reaction include enzymes for regenerating energy in the reaction system and enzymes for hydrolyzing inorganic pyrophosphoric acid formed during the transcription or translation reaction.

Please amend paragraph [0012] as follows:

[0012] In the present invention, the combination of first and second substances, i.e., mutually interacting in affinity chromatography can be selected from among a combination of a protein or a peptide fragment with a metal ion, a combination of an antigen with an antibody, a combination of a protein with a protein or a peptide fragment, a combination a of protein with a specific low-molecular weight compound selected from the group consisting of amino acids, DNAs, dyes, vitamins and lectins, a combination of a protein with a saccharide and a combination of a protein or a peptide fragment with an ion exchange resin. Among all, it is favorable to use a combination of histidine tag with a metal chelate such as a nickel complex or a cobalt complex taking advantage of a bond between a protein or a peptide fragment and a metal ion.

Please amend paragraph [0013] as follows:

[0013] The first and second substances adhering to each other usable in the present invention are not restricted to a combination of substances mutually interacting in affinity chromatography. Use may be made therefor of, for example, substances magnetically adhering to each other too.

Please amend paragraph [0014] as follows:

[0014] The present invention further relates to a kit of protein components for a reaction system for producing a peptide or a peptide derivative by transcribing a DNA into an RNA and then translating the RNA produced or translating an RNA *in vitro* wherein ~~characterized in that~~ the kit comprises a part or all of protein components constituting the transcription/translation reaction system and that the protein components are selected from the group consisting of enzymes and factors which are labeled with one of a pair of substances adhering to each other. In this kit, the protein components are selected from the factors and enzymes for the transcription or translation reaction and other enzymes required in the constitution of the reaction system. Particular examples of the factors and enzymes for the transcription or translation reaction include initiation factors, elongation factors, termination factors, aminoacyl-tRNA synthetase, methionyl-tRNA transformylase and RNA polymerase. Particular examples of the enzymes required in the constitution of the reaction system other than the factors and enzymes for the transcription or translation reaction include enzymes for regenerating energy in the reaction system and enzymes for hydrolyzing inorganic pyrophosphoric acid formed during the transcription or translation reaction. The kit of protein components according to the present invention may

comprise an adsorbent for capturing the protein components labeled with one of a pair of the substances adhering to each other.

Please amend paragraph [0015] as follows:

[0015] The kit of protein components according to the present invention may comprise combinations, different from each other, of the first and second substances, wherein the first substance is used for labeling a part or all of the protein components constituting the reaction system and ~~with~~ the second substance is used as an adsorbent for capturing the ~~labeled~~ protein components labeled by the first substance.

Please amend paragraph [0018] as follows:

[0018] The factors and enzymes for the transcription or translation reaction are not restricted to those originating in prokaryotic cells such as *E. coli* but use can be made of those originating in eukaryotic cells. In case (1) of translating an RNA, these factors and enzymes include initiation factors, elongation factors, termination factors, 20 aminoacyl-tRNA synthetases, and tRNAs attached to natural or unnatural amino acids, and methionyl-tRNA transformuylase is further included in an *E. coli*-origin in vitro reaction system. In case (2) of transcribing a DNA into an RNA and then translating the RNA

produced, these factors and enzymes include, in addition to those cited in the above case (1), RNA polymerase such as T7RNA polymerase. The translation reaction can be regulated by eliminating the termination factors from the reaction system of the above-described case (1) or (2), as will be discussed hereinafter.

Please amend paragraph [0025] as follows:

[0025] On the contrary to these conventional methods, the present inventors have introduced one of a pair of first and second substances adhering to each other not into the target peptide but protein components constituting the *in vitro* peptide synthesis system, based on a novel finding that the transcription or translation reaction can proceed even though the factors and enzymes for the transcription or translation and other enzymes are labeled with one of a pair of the substances adhering to each other.

Please amend paragraph [0026] as follows:

[0026] The combination of a pair of the first and second substances adhering to each other to be used in the present invention may be an arbitrary one, so long as the transcription or translation reaction is not disturbed thereby. Although the adhesion of these substances to each other may be either reversible

or irreversible, it is preferable to use a pair of substances which reversibly ~~irreversibly~~ adhere to each other. This is because the protein components constituting the reaction system can be repeatedly used in such a case.

Please amend paragraph [0027] as follows:

[0027] As an example of the combination of the first and second substances adhering to each other, citation may be made of a combination of an adsorption column with a substance capable of selectively binding to the adsorption column. Typical examples thereof include substances mutually interacting in affinity chromatography. For example, a metal complex such as a nickel or cobalt complex serves as a ligand of an adsorption column while histidine tag serves as a substance capable of selectively binding to the adsorption column. Moreover, use can be made of combinations of various ligands with substances capable of selectively binding thereto as will be discussed hereinafter, so long as the reaction is not disturbed thereby. That is to say, the combination of the first and second substances mutually interacting in affinity chromatography usable in the present invention can be selected from among, for example, a combination of a protein or a peptide fragment with a metal ion, a combination of an antigen with an antibody, a combination of a protein with a protein or a peptide

fragment, a combination of a protein with a specific low-molecular weight compound selected from the group consisting of amino acids, DNAs, dyes, vitamins and lectins, a combination of a protein with a saccharide and a combination of a protein or a peptide fragment with an ion exchange resin.

Please amend paragraph [0028] as follows:

[0028] The first and second substances adhering to each other usable in the present invention are not restricted to a combination of substances mutually interacting in affinity chromatography but can be arbitrarily selected depending on the purpose. For example, use may be made therefor of substances magnetically adhering to each other. As an example thereof, a combination of a magnetic bead-labeled protein with a magnet may be cited. In this case, protein components constituting the peptide synthesis system, which have been individually labeled with the magnetic beads, can be adsorbed by the magnet and thus captured.

Please amend paragraph [0029] as follows:

[0029] In the present invention, the adsorbent (i.e., the second substance) is used in the form of, for example, a column, a matrix, a filter or a bead. Alternatively, it may be fixed to a carrier (support), if desired. To fix the adsorbent to the carrier, an

appropriate means can be selected from among known techniques depending on the properties of the adsorbent.

Please amend paragraph [0030] as follows:

[0030] There are a plural number of combinations of the first substance for labeling a part or all of the protein components constituting the reaction system with the second substance used as the adsorbent for capturing the ~~thus labeled~~ protein components thus labeled by the first substance. It is possible to use such combinations differing from each other in a single reaction system. It may be rather considered as favorable to select the most suitable labels for respective protein components and then select adsorbents appropriate for these labels.

Please amend paragraph [0031] as follows:

[0031] Since the factors and enzymes for the transcription/translation reaction system and other enzymes are labeled with one of a pair of the first and second substances adhering to each other in the present invention, the protein components constituting the reaction system can be obtained each in a highly pure state and the reaction system is not contaminated with any unknown and unnecessary or inhibitory components. Thus, the reaction system can be established and, consequently, the

reaction efficiency can be largely elevated. In addition, it becomes possible to quickly separate the target peptide from these reaction constituents after synthesizing the peptide. In the conventional cell-free systems, a peptide formed by the reaction is purified by extraction. It is therefore needed to select an appropriate purification procedure in each case depending on the physical and chemical properties of the reaction product. In the present invention, in contrast thereto, the components constituting the reaction system are eliminated by using the adsorbent (i.e., the second substance) and the reaction product is thus purified. Accordingly, it is theoretically possible to apply the same purification procedure to any reaction products regardless of the physical and chemical properties thereof. In addition, the target peptide thus obtained has a very high purity.

Please amend paragraph [0052] as follows:

[0052] Among the constituents of the reaction system as described in the above (3) and (4), protein components are expressed in a large amount in *E. coli* (for example, commercially available *E. coli* BL21 strain) in the form of fused proteins (for example, His-tagged proteins) labeled at the N- or C-terminus with one of a pair of the first and second substances adhering to each other as will be described in greater detail hereinafter. Then these proteins

thus expressed are purified by using ~~a nickel column connected to~~ an adsorbent such as a nickel column containing the other substance such as fast protein liquid chromatography (FPLC) and then supplied to the reaction system. In addition to *E. coli*, it is possible to express these proteins in animal cells, yeasts, *Bacillus subtilis* or the like. Alternatively, it is possible to produce these proteins by using an *in vitro* peptide synthesis system.

Please amend the heading of paragraph [0053] as follows:

(5) Label and adsorbent, i.e., a pair of first and second substances adhering to each other

Please amend paragraph [0053] as follows:

[0053] In the present invention, all or a part of the protein components constituting the reaction system as described in the above (3) and (4) are labeled with one of a pair of substances adhering to each other and the thus labeled protein components are captured by using the other substance as an adsorbent to thereby isolate the target peptide formed in the reaction system. As typical examples of a pair of the first and second substances adhering to each other, substances mutually interacting in affinity chromatography can be cited. However, any pair of first and second substances adhering to each other are usable in the present

invention without restriction to substances mutually interacting in affinity chromatography, so long as these substances can be used in capturing the protein components.

Please amend paragraph [0054] as follows:

[0054] Proteins exert physiological effects via specific mutual interactions with certain substances. Adsorption chromatography which is carried out by taking advantage of such a specific interaction (affinity) between a protein and a certain substance (ligand) is called affinity chromatography. Examples of a combination of the first and second substances adhering specifically to each other include a protein or a peptide fragment with a metal ion or a chelate compound, an antigen with an antibody, a cytokine or a hormone with a receptor, and an enzyme with a substrate or an inhibitor. Furthermore, specific amino acids, DNAs, dyes, vitamins, lectins and the like mutually bind to proteins having affinities therefor respectively.

Please amend paragraph [0055] as follows:

[0055] One of the substances of such a combination is fixed as a ligand to a carrier or a support to form an adsorbent. Then materials labeled with the other substance (the protein components constituting the reaction system in the case of the present

invention) are passed therethrough. Thus, the label (the first substance) specifically binds to the ligand (the second substance). Affinity chromatography based on this specific binding has been commonly employed as a means of purifying proteins. Various carriers have been marketed by a number of manufacturers, which makes this means highly available. In purifying a protein using an antigen-antibody reaction, for example, use is made of a combination of an antigen determinant (an epitope) having a known structure with an antibody specific to the epitope. There have been marketed various combinations of vectors and adsorbents for carrying out this means. In a preferred embodiment of the present invention, use is made of a combination of such substances adsorbing to each other which are employed in affinity chromatography. In preparing the labeled protein components to be used in the present invention, the label is useful in the purification. It is also possible to label the protein components with a plural number of labels at the same time. In this case, it is possible that the label usable for the purification in the production process is cut off while other labels are used for the separation of the target product formed in the *in vitro* peptide synthesis system.

Please amend paragraph [0056] as follows:

[0056] Now, embodiments of the present invention will be illustrated by citing some examples of the combination of the first and second substances adhering to each other. However, it is to be understood that the present invention is not construed as being restricted these examples.

Please amend paragraph [0108] as follows:

[0108] To obtain a template for MFL mRNA, AUGUUCUUGUAA (SEQ ID NO:4), a DNA ~~sequence~~ (translated into fMet-Phe-Leu-Stop; formylmethionine-phenylalanine-leucine-stop codon; hereinafter referred to simply as MFL) was constructed as follows. An oligonucleotide A: 5'-Tatgttcttgtaac (SEQ ID NO:5) was annealed with another oligonucleotide B: 5'-TCGAgttacaagaaca (SEQ ID NO:6) to give a double-stranded DNA containing NdeI and XhoI sequences. Next, this DNA was cloned into the NdeI and XhoI sites of a plasmid vector pET29a (Novagen). The resultant plasmid was transcribed as in the above-described case of DHFR gene.